REMARKS

Review and reconsideration of the Office Communication of January 2, 2003, is respectfully requested in view of the following remarks.

Applicants thank the Examiner for the indication that Claims 27-34 contain allowable subject (subject only to formalities rejections). Applicants have re-written Claims 27 and 29 in independent form.

The Claims have been amended to overcome the formalities rejection. No new matter has been added to the claims.

Applicants would like to point out to the Examiner that somatic means that the soma (cell) is altered. Transgenesis means that foreign genes transfect the host cell. Somatic transgenesis (as in Claim 23) means an approach in which a living host (entire organism) is transformed with a foreign episomal DNA and that transformation is limited to a part of an organism, certain cells or certain tissue/organs. **The effect is based on deposition of genes and not on heterologous production of proteins. More precisely following somatic transgenesis, the proteins are produced inside the eukaryotic cell and NOT by the gene delivery vehicle followed by a delivery of protein into the host cell.**

Applicants reviewed the Goossens reference and note that the main difference between the reference and Claim 23 of the present invention is that the reference fails to teach a

bacteria useful as a vehicle for **gene transport** and **gene transfer.**

Applicants note that Goossens et al teaches Listeria monocytogenes carrying a heterologous gene under the control of a prokaryotic regulatory sequence and can be used in an animal model to elicit an immune response against the corresponding **protein.** This is, however, not subject of the present patent application. The present invention teaches that Listeria monocytogenes or other intracellular vital bacteria can be used to transport **genes** into eucaryotic cells. The TGC method (present invention) uses the intracellular bacteria solely as a transport vehicle for genes. There is no expression of the target gene in the bacteria.

Applicants reviewed the Branstrom et al (WO 97/08955) reference and note that the main difference between the reference and Claim 23 of the present invention is that the present invention uses live gene carriers to transfer target organs far from the site of administration. Branstrom et al. teach that attenuated Shigella flexneri can be used to transfer plasmid DNA to mucosal epithelial cells. The bacteria are attenuated in such a way, that they **die** once they reached the cytoplasm as to avoid the induction of disease.

Three main points distinguish the TGC method from the method of Branstrom et al.:

1. It is a hallmark of Shigella as used by Branstrom et al, that the bacteria induce no systemic but only a localized infection. Shigella infects the epithelial

> mucosa via M-cells and can then only spread to neighbouring cells and macrophages, thus leading to typical gastrointestinal symptoms (Sansonetti 2001, Am. J. Physiol. Gastrointest. Liver Physiol. 280: G319). Shigella is not able to infect other organs but the gut. Thus, it is characteristic for the method of Branstrom et al. that following oral application, the bacteria carrying the target genes can only transfect the epithelial mucosa (compare Fig. 2).

It was therefore the task of the present invention to establish a bacterial transfection system that is able to transfect tissues and organ far from the site of application. The inventors solved this problem by using bacteria, which induces systemic infections, for example L. monocytogenes (Fig. 2). When applied orally, L. monocytogenes enters the host organism also in the gut via M-cells, but subsequently spreads to infect tissues as diverse and far away as the brain or the udder (Vázquez-Boland et al. Clin. Microbiol. Rev. 2001:584-640). Thus, the use of bacteria inducing a systemic infection in the present invention adds a whole new perspective to DNA transfer via bacteria: gene carriers can be applied orally and can then transfect any organ of choice. Gene carriers, according to Branstrom et al., cannot be used in such a way, as the infection is locally restricted.

2. It is an object of the invention of Branstrom et al.

> "... to provide an attenuated strain of Shigella which

retains the ability to enter a cell, but dies once inside the cell." (Page 6). The TGC method does not work with such bacteria; it is central to the TGC method that the "**gene carriers**" retain its potential to invade, survive in and to multiply in **eucaryotic cells,** and moreover to infect neighbouring cells. By using live bacteria, the TGC method ensures an amplification of the transferred DNA, ascertains that the cells of the targeted organ are transfected, and thus avoids the need for repeated applications, as is necessary when using the gene carriers proposed by Branstrom et al.

3. The method of Branstrom et al. functions with dead bacteria (Page 4: "The bacteria used in this delivery system do not have to be alive in order to deliver the nucleic acids of choice."). Thus, Branstrom et al. use the bacteria solely to transport the DNA across the eucarotic cell membrane.

The method of Branstrom et al can be compared to traditional methods such as transfection by polycationic lipids or electroporation. In contrast, the TGC method does only work with live bacteria; it is not functional with dead bacteria. While dead bacteria are taken up by the eucaryotic host cells, they cannot spread to neighbouring cells and far away tissues, thus the quintessential feature of the TGC method cannot be achieved using dead bacteria. It is the new and innovative element of the present invention to use the full

pathophysiological potential of the bacteria, that is their inherent properties to infect and grow within eucaryotic cells.

Finally, Applicants would like to point out to the Examiner that the Dietrich et al. reference was published on the 16th of January 1998, while the priority date of the patent application is the 11th of December 1997. Thus, Dietrich et al. cannot be considered as prior art against the present invention.

Paragraph 1

The Examiner indicates that Applicants' amendment received on 7/8/02 has been entered. Further, Claim 31 has been amended, Claims 23-51 are pending, and Claims 23-34 are under current consideration.

Paragraph 2 (Election/Restrictions)

The Examiner indicated that Claims 35-51 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a non-elected invention, there being no allowable generic or linking claim. Applicants timely traversed the restriction (election) requirement in Paper No. 9.

Paragraph 3 (Priority)

The Examiner acknowledges Applicants' claim for foreign priority based on an application filed in Germany on 12/11/97. However, the Examiner notes that Applicants have not filed a certified copy of the German application.

In response, Applicant will be filing a certified copy of the priority document shortly.

Paragraphs 4 - 13 (Formalities)

The Examiner indicated that the previous formalities rejections were withdrawn.

Applicants are pleased for the indication.

The Examiner rejects Claims 27-30 under 35 USC § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The position of the Examiner can be found on pages 3-5 of the Office Action.

In response, Applicants have amended the claims to overcome the formality rejection.

Applicants would also like to point out to the Examiner that Claims 27 and 29 are only specialized examples for the method described in claim 23.

Claim 23 describes a method for transfecting eucaryotic cells using prokaryotic "gene carriers." Claims 27 and 29 give examples of so called "suicide gene carriers," meaning these gene carriers die without administration of antibiotics or other outside factors.

The inventors provided the concept of suicide gene carriers, that is bacteria that infect the host animal and die once their target cells are reached. In order to generate such suicide gene carriers, they had to identify genes, which can be

mutated or deleted such that the mutation has a lethal effect on the bacteria.

In L. monocytogenes, the gene that the inventors identified was gene dapE. The gene codes for the protein N-succinyl-L,L-DAP-desuccinylase, an enzyme which is important for the biosynthesis of meso-diaminopimelate, an essential building block of the cell wall of grampositive and -negative bacteria. The sequence of this protein was first described in 1992 in E. coli (Bouvier et al. 1992, J. Bacteriol. 174: 1622-1627). Those skilled in the art know that to perform its enzymatic functions, an enzyme needs characteristic structures such as the enyzmatic pocket and catalytically active aminoacids. For the DapE protein, some of these structures were identified before the present application was filed (Meinnel et al. 1992, J. Bacteriol. 174: 2323-2331). Subsequent work revealed that the protein N-succinyl-L,L-DAP-desuccinylase from different bacteria is so highly conserved that recently, a conserved protein domain could be formulated (Conserved Domain Database at the NIH; CD: COG0624).

What is claimed in the present application is a gene, dapE, and its variants that perform the defined function of N-succinyl-L,L-DAP-desuccinylase. As the aminoacids necessary for this enzymatic activity were known before the present application was filed, those skilled in the art could derive variants of the dapE gene with a 35% homology to SEQ-ID NO 1 without undue experimentation based on the published data and the information provided in the description. Therefore, the

written description, in combination with the state of the art, convey to anyone skilled in the art that the inventors were, in fact, in possession of what is claimed.

The second gene the inventors provided for the construction of suicide gene carriers is gene cspL of L. monocytogenes. At the time the application was filed, cold shock proteins (CSP) had been identified in a great number of bacteria, amongst these B. cereus, B. subtilis, H. influenzae and E. coli (compare Graumann et al. 1996, Arch. Microbiol. 166: 293, and references therein), and also the threedimensional structure of two CSPs had been determined (B. subtilis and E. coli, compare Graumann et al.). These analyses revealed that CSPs consist of a group of proteins with high structural and sequential homologies. Characteristically CSPs contain a so-called cold-shock domain with a typical betabarrel fold. Thus, at the time the application was filed, the characteristic features of CSPs were known to anyone skilled in the art. Based on SEQ-ID NO 2 and the state of the art, it is possible for anyone skilled in the art to generate without any undue experimentation cspL mutants with a homology of at least 35% that fulfill the structural restrains of the cold-shock domain. Thus, the Applicants provided the necessary common features of cspL and its variants and were therefore in possession of what is claimed.

Accordingly, withdrawal of the rejections is respectfully requested.

Paragraphs 14 - 17 (Formalities)

The Examiner rejects Claims 23-34 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter, which Applicants regard as the invention.

The Examiner indicates that Claim 23 is indefinite as written. The claim, as written, recites that expression of a foreign DNA as being under the control of a eukaryotic regulator gene. Neither the specification nor the prior art of record have provided a definition of a eukaryotic regulator gene that can control expression of a DNA sequence. It would appear that Applicants intended to recite that expression of a foreign DNA sequence is controlled by a eukaryotic regulatory sequence such as a promoter. Correction is required. Claims 24-34 depend from Claim 23.

In response, Applicants have amended the claim as suggested by the Examiner to overcome the rejection.

Accordingly, withdrawal of the rejections is respectfully requested.

Paragraphs 18 - 28 (Anticipation)

The Examiner rejects Claims 23-24, 26, and 34 under 35 U.S.C. 102(b) as being anticipated by Goossens et al (International Immunology, 1995, 7(5): 797-805).

The position of the Examiner can be found on page 7 of the Office Action.

Applicants respectfully traverse.

For a reference to anticipate, it must disclose all the elements of the claim.

Applicants reviewed the reference and note that the main difference between the reference and Claim 23 of the present invention is that the reference fails to teach a bacteria useful as a vehicle for **gene transport** and **gene transfer.**

Applicants note that Goossens et al teaches Listeria monocytogenes carrying a heterologous gene under the control of a prokaryotic regulatory sequence can be used in an animal model to elicit an immune response against the corresponding **protein.**

This is, however, not subject of the present patent application. The present invention teaches that Listeria monocytogenes or other intracellular vital bacteria can be used to transport **genes** into eucaryotic cells. The TGC method (present invention) uses the intracellular bacteria solely as a transport vehicle for genes. There is no expression of the target gene in the bacteria.

The attached Figure 1 illustrates the two concepts. The TGC methods aims at transferring selected genes to render the cells or tissues transgenic while Goosens et al. transfer proteins to evoke an immune response. Thus, the teachings of Goosens et al. did not anticipate the TGC method, as Goosens et al. used a totally different technology.

The Examiner rejects Claims 23-24 and 26 under 35 U.S.C. 102(b) as being anticipated by Branstrom et al (WO 97/08955).

The position of the Examiner can be found on page 8 of the Office Action.

Applicants respectfully traverse.

For a reference to anticipate, it must disclose all the elements of the claim.

The TGC method (present invention) is a technology that uses live gene carriers to transfer target organs far from the site of administration. Thus, the TGC method differs from the works of Branstrom et al substantially. Branstrom et al. teach that attenuated Shigella flexneri can be used to transfer plasmid DNA to mucosal epithelial cells. The bacteria are attenuated in such a way, that they die once they reached the cytoplasm as to avoid the induction of disease. Three main points distinguish the TGC method from the method of Branstrom et al.:

1. It is a hallmark of Shigella as used by Branstrom et al., that the bacteria induce no systemic, but only a localized infection. Shigella infects the epithelial mucosa via M-cells and can then only spread to neighbouring cells and macrophages, thus leading to typical gastrointestinal symptoms (Sansonetti 2001, Am. J. Physiol. Gastrointest. Liver Physiol. 280: G319). Shigella is not able to infect other organs but the gut. Thus, it is characteristic for the method of Branstrom et al. that following oral application, the bacteria carrying the target genes can only transfect the epithelial mucosa (compare Fig. 2).

It was, therefore, the task of the present invention to establish a bacterial transfection system that is able to transfect tissues and organs far from the site of application. The inventors solved this problem by using bacteria, which induces systemic infections, for example L. monocytogenes (Fig. 2). When applied orally, L. monocytogenes enters the host organism also in the gut via M-cells, but subsequently spreads to infect tissues as diverse and far away as the brain or the udder (Vázquez-Boland et al. Clin. Microbiol. Rev. 2001:584-640). Thus, the use of bacteria inducing a systemic infection in the present invention adds a whole new perspective to DNA transfer via bacteria: gene carriers can be applied orally and can then transfect any organ of choice. Gene carriers, according to Branstrom et al., cannot be used in such a way, as the infection is locally restricted.

2. It is an object of the invention of Branstrom et al. "... to provide an attenuated strain of Shigella which retains the ability to enter a cell, but dies once inside the cell." (Page 6). The TGC method does not work with such bacteria; it is central to the TGC method that the "**gene carriers**" retain its potential to invade, survive in, and to multiply in **eucaryotic cells,** and moreover, to infect neighbouring cells. By using live bacteria, the TGC method ensures an amplification of the transferred DNA, ascertains that the cells of the targeted organ are transfected, and thus avoids the need for repeated applications, as is

necessary when using the gene carriers proposed by Branstrom et al.

3. The method of Branstrom et al. functions with dead bacteria (Page 4: "The bacteria used in this delivery system do not have to be alive in order to deliver the nucleic acids of choice."). Thus, Branstrom et al. use the bacteria solely to transport the DNA across the eucarotic cell membrane.

The method of Branstrom et al. can be compared to traditional methods such as transfection by polycationic lipids or electroporation. In contrast, the TGC method does only work with live bacteria; it is not functional with dead bacteria. While dead bacteria are taken up by the eucaryotic host cells, they cannot spread to neighbouring cells and far away tissues; thus, the quintessential feature of the TGC method cannot be achieved using dead bacteria. It is the new and innovative element of the present invention to use the full pathophysiological potential of the bacteria, that is their inherent properties to infect and grow within eucaryotic cells.

The Examiner rejects Claims 23-24, 26, and 34 under 35 U.S.C. 102(b) as being anticipated by Dietrich et al (Nature Biotechnology, 1998, 16: 181-185).

The position of the Examiner can be found on page 8 of the Office Action.

Applicants respectfully traverse.

Applicants would like to point out to the Examiner that the Dietrich et al. reference was published on the 16th of January

1998, while the priority date of the patent application is the 11th of December 1997. Thus, Dietrich et al. cannot be considered as prior art against the present invention.

Accordingly, withdraw of the rejections is respectfully requested.

Paragraph 29 (Allowable subject matter)

The Examiner indicated that Claims 27-34 appear to be free of the prior art of record (contain allowable subject matter).

Applicants would like to thank the Examiner for the indication.

Applicants have re-written Claims 27 and 29 in independent form.

Favorable consideration and early issuance of the Notice of Allowance are respectfully requested. Should further issues remain prior to allowance, the Examiner is respectfully requested to contact the undersigned at the indicated telephone number.

Respectfully submitted,

Evelyn A. Defillo

Evelyn A. Defillo
Registration No. 45,630

PENDORF & CUTLIFF
5111 Memorial Highway
Tampa, FL 33634-7356
(813) 886-6085

Date: **April 30, 2003**

CERTIFICATE OF MAILING AND AUTHORIZATION TO CHARGE

I hereby certify that the foregoing AMENDMENT C for U.S. Application No. 09/581,005 filed June 6, 2000, was deposited in first class U.S. mail, postage prepaid, addressed: Attn: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on **April 30, 2003**.

The Commissioner is hereby authorized to charge any additional fees, which may be required at any time during the prosecution of this application without specific authorization, or credit any overpayment, to Deposit Account No. 16-0877.

Evelyn A. Defilló

Evelyn A. Defilló